Final Scientific Report

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BARD Project Number: CB-9002-04

Date of Submission of the report: 16th March, 2008

Project Title: Identification and gene regulation of the desaturase enzymes involved in sex-pheromone biosynthesis of pest moths infesting grain.

Investigators	**Institutions**
Principal Investigator (PI): Ada Rafaeli	ARO, Volcani Center
Co-Principal Investigator (Co-PI): Wendell Roelofs	Geneva, USA
Collaborating Investigators: Anat Zada	ARO, Volcani Center

Keywords:

Budget: IS: $140,000 US: $10,000 (+ Postdoctoral Fellow as matching fund) Total: $150,000

Signature
Principal Investigator

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Signature
Authorizing Official, Principal Institution

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Abstract

The **original objectives** of the approved proposal included: 1. Establishment of the biosynthetic pathways for pheromone production using labeled precursors and GC-MS. 2. The elucidation of a circadian regulation of key enzymes in the biosynthetic pathway. 3. The identification, characterization and confirmation of functional expression of the delta-desaturases. 4. The identification of gene regulatory processes involved in the expression of the key enzymes in the biosynthetic pathway. **Background to the topic:** Moths constitute one of the major groups of pest insects in agriculture and their reproductive behavior is dependent on chemical communication. Sex-pheromone blends are utilized by a variety of moth species to attract conspecific mates. The sex pheromones used are commonly composed of blends of aliphatic molecules that vary in chain length, geometry, degree and position of double bonds and functional groups. They are formed by various actions of specific delta-desaturases to which chain shortening, elongation, reduction, acetylation, and oxidation of a common fatty acyl precursor is coupled. In most of the moth species sex-pheromone biosynthesis is under circadian control by the neurohormone, PBAN (pheromone-biosynthesis-activating neuropeptide). The development of specific and safe insect control strategies utilizing pheromone systems depends on a clear knowledge of the molecular mechanisms involved. In this proposal we aimed at identifying and characterizing specific desaturases involved in the biosynthetic pathway of two moth pest-species of stored products, *P. interpunctella* and *S. cerealella*, and to elucidate the regulation of the enzymes involved in pheromone biosynthesis. Due to technical difficulties the second stored product pest was excluded from the study at an early phase of the research project. **Major conclusions:** Within the framework of the planned objectives we confirmed the pheromone biosynthetic pathway of *P. interpunctella* and *H. armigera* by using labeled precursor molecules. In addition, in conjunction with various inhibitors we determined the PBAN-stimulated rate-limiting step for these biosynthetic pathways. We thereby present conclusive evidence that the enzyme Acetyl Coenzyme A Carboxylase is activated as a result of PBAN stimulation. We also found that *P. interpunctella* produce the main pheromone component *Z*9, *E*12 Tetradecenyl acetate through the action of a Δ11 desaturase working on the 16:Acid precursor. This is evidenced by the high amount of incorporation of ^{2}H-labeled 16:Acid into pheromone when compared to the incorporation of ^{2}H-labeled 14:Acid. However, in contrast to reports on other moth species, *P. interpunctella* is also capable of utilizing the 14:Acid precursor, although to a much lesser extent than the 16:Acid precursor. Despite the discovery of nine different desaturase gene transcripts in this species, from the present study it is evident that although PCR detected all nine gene transcripts, specific to female pheromone glands, only two are highly expressed whereas the other 7 are expressed at levels of at least 10^{5} fold lower showing very low abundance. These two genes correspond to Δ11-like desaturases strengthening the hypothesis that the main biosynthetic pathway involves a Δ11 desaturase.

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Achievements

Significance of main scientific achievements or innovations.

In the present study we combined the use of labelled precursors with enzyme inhibitors to decipher the biosynthetic pathway of pheromone biosynthesis and the rate-limiting step/s that are regulated by Pheromone-Biosynthesis-Activating-Neuropeptide (PBAN). We demonstrated that *Plodia interpunctella* is able to utilize hexadecanoic acid, and to a lesser extent tetradecanoic acid, for the biosynthesis of the main pheromone component (*Z*,*E*)-9,12-tetradecadienyl acetate. This indicated that the main pathway involves a *Δ*11 desaturase, chain shortening, followed by a *Δ*12 desaturase, but that a functional *Δ*9 desaturase could also be utilized. In *P. interpunctella* nine different desaturase encoding transcripts have been isolated as potential desaturase genes, which may produce the desaturase enzymes involved in pheromone biosynthesis however, the functionality of these genes has not been determined. Using reverse transcription-quantitative real-time polymerase chain reaction (RT-QPCR) we distinguish two out of nine possible desaturase gene transcripts in *P. interpunctella* that are expressed at the highest levels. The effect of PBAN on the different steps in the biosynthetic pathway has been investigated in several lepidopteran species but the key rate-limiting enzymes involved have not been conclusively established. A particular enzyme within the pheromone biosynthetic pathway that is regulated by PBAN has not yet been conclusively identified and there appears to be no particular pattern as to which enzyme within the pheromone biosynthetic pathway will be regulated by PBAN. Thus in this study rate limiting step for PBAN stimulation was studied in two moth species so as to compare the biosynthesis of a diene *(P. interpunctella*) and a monoene *(Helicoverpa armigera)* main pheromone component. In both species incorporation of label from the ^{13}C sodium acetate precursor was activated by PBAN whereas no stimulatory action was observed in the incorporation of the precursors: ^{13}C malonyl coenzyme A; hexadecanoic 16,16,16-$^{2}H_3$ or tetradecanoic 14,14,14-$^{2}H_3$ acids. The Acetyl Coenzyme A Carboxylase (ACCase) inhibitor, Tralkoxydim, inhibited the PBAN-stimulation of incorporation of stable isotope whereas the fatty acyl reductase inhibitor, Mevastatin, failed to influence the stimulatory action of PBAN. These results provide irrefutable support to the hypothesis that PBAN affects the production of malonyl coenzyme A from acetate by the action of ACCase in the pheromone glands of these moths.

Agricultural and/or economic impacts of the research findings.

Our progress and achievements in this project have advanced our knowledge concerning the rate-limiting steps in the biosynthetic pathways of moth pest species and will pave the way to the elucidation of methodologies targeted at silencing specific regulatory pathways. It is still a mystery as to why so many desaturase gene-transcripts are present and what factors regulate their functionality. Whether the highly expressed genes determined in this study represent the functional genes awaits further characterization of full gene sequences and appropriate functional expression studies. The research launches opportunities for studying molecular regulation pathways and the evolution of desaturases. Overall this research will advance research towards the goal of finding specific methodologies for disrupting sex-pheromone production of pest moths.

Details of cooperation

The insect culture was maintained in the Israeli PI's laboratory. A cDNA library of pheromone glands was prepared in the Israeli PI's laboratory and initially brought to Cornell by an MSc student, Oren Tsfadia who spent 2 months, during the summer to train in Cornell. The Cornell laboratory continued to provide advice on the molecular biological aspects of the proposal (Dr. Bingye Xue as postdoctoral fellow and Prof. Wendell Roelofs as Co-PI). The biosynthetic pathway experiments were conducted in the pheromone research laboratory of Prof. Rafaeli (Israeli PI's laboratory) by Mr. Tsfadia and samples, ready for GC/MS analyses were taken to the CI: Dr. Anat Zada's laboratory (Chemistry Unit, ARO), where all the GC/MS identifications were supervised. Constant consultations (via e-mail) between the PI and CI's enabled co-ordination of all the duties that were assigned. Three International Conferences (the Society for Chemical Ecology, held in Barcelona, 2006 and Jena, 2007 and the European Congress of Entomology, held in Izmir, 2006) served as an opportunity for meetings between the PI and Co-PI to consolidate our research plans and strategies.

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	1			1
Submitted, in review, in preparation				
Invited review papers				
Book chapters				
Books				
Master theses	1			1
Ph.D. theses				
Abstracts				
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings		1 (2 months)	3	4
Longer Visits (Sabbaticals)				

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

List of publications:

Reviewed Journals:

Tsfadia, O., Azrielli, A., Falach, L., Zada, A., Roelofs, W., Rafaeli, A. (2008) Pheromone Biosynthetic Pathways: PBAN-Regulated Rate-Limiting Steps and Differential Expression of Desaturase Genes in Moth Species. *Insect Biochemistry and Molecular Biology (in press)* doi:10.1016/j.ibmb.2008.01.005

Student Theses:

Oren Tsfadia, MSc (Plant Protection) (2007) Pheromone biosynthesis and PBAN regulation in two Lepidopteran species, *Plodia interpunctella* (Pyralidae) and *Helicoverpa armigera* (Noctuidae): Rate-limiting steps in the biosynthetic pathway. The Hebrew University, Faculty of Agriculture, Food and Environmental Quality Sciences, Rehovot, Israel.

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